IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No. 24741-1505

In re patent application of:

SIMON, Jan *et al.*

Confirmation No.: 3688

Application No.: 09/763,794

Group Art Unit: 1642

Filed: July 12, 2001

Examiner: C. Yaen

For: LOW MOLECULAR WEIGHT FRAGMENTS OF HYALURONIC ACID FOR PRODUCING VACCINES

H 18
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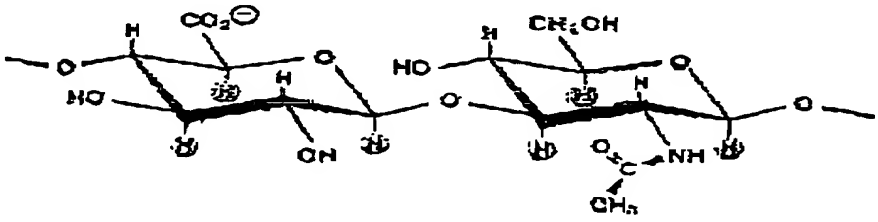
9/2003

Declaration under 37 C.F.R. § 1.132

1. I, Prof. Dr. Simon, am a co-inventor of the captioned application and I have read and understood the Office Action issued February 12, 2003, and the art cited therein.

2. As shown below, hyaluronic acid is composed of repeating disaccharide-units of [glucuronic-acid (β 1-3)-N-acetyl-glucosamine (β 1-4)]$_n$. Proteins can bind on this molecule at the free aldehyde group within the N-acetyl-glucosamine molecule or after reduction and cleavage of the end-standing ring.



3. Low molecular weight hyaluronic acid fragments were generated by enzymatic digestion of high molecular weight hyaluronic acid with bovine testis hyaluronidase for 12 h in 1 M Na-acetate buffer pH 5.0 at 37°C. The fragments were separated on a Biogel P10

(BioRad) 3.5 x 115 cm- gass-column overnight. Samples were collected from the column with a Pharmacia LKB Frac. 100 fraction collector for 12 h on 0 min/each. Low molecular weight hyaluronic acid fragments were adjusted to a concentration of 1 mg/ml for further experiments.

4. Wenge et al. claims a method that uses hyaluronic acid of 750,000 Da size. The low molecular weight hyaluronic acid fragments of 2-12 disaccharide are much smaller, i.e. their size is 2,000-5,000 Da size.

5. Noble et al. describes a method that uses hyaluronic acid fragments of 200,000 Da size. By contrast, the low-molecular weight oligosaccharides of Hyaluronan of 2-12 disaccharide length claimed in the captioned application are of 2000-5000 Da size.

6. Importantly, we have shown high molecular weight hyaluronic acid such as those preparations claimed by Wenge et al. and Noble et al. to be completely ineffective to stimulate dendritic cells. We discuss these findings below:

7. Specifically, human monocyte-derived dendritic cells were cultured for 48 hours in medium with 30 μg/ml of different fragment concentrations, all prepared from endotoxin-free, high molecular weight (500-100 kDa size) hyaluronic acid (HMW-HA). The HMW-HA was sonified to generate intermediate sized hyaluronic acid fragment of 300-60 kDa size (INT-HA). Finally, low molecular weight oligosaccharides were generated by enzymatic digestion and gel column separation (2-5 kDa) (sHA).

8. In the attached Figure, the numbers behind the sHA indicate the number of disaccharide units (size) of the oligosaccharides (filled bars). Dendritic cells were then stained with monoclonal antibodies directed against surface markers.

9. In the Figure, the results are shown as mean fluorescence intensities (MFI). Importantly, only sHA, including hyaluronic acid fragments of 2-12 disaccharide length induce phenotypical changes in dendritic cells that can be correlated with dendritic cell maturation. The INT-HA and HMW-HA fragments, including those fragments disclosed by Wenge et al. and Noble et al. did not induce phenotypical changes in dendritic cells.

10. Accordingly, intermediate sized fragments of 300,000 - 60,000 Da size, including fragment lengths reported by Noble et al. and large sized fragments of 750,000 Da described by Wenge et al., are ineffective to stimulate dendritic cells. Thus, based on Nobel et al. and Wenge et al., a person of ordinary skill would not have been guided to use low-molecular weight hyaluronic acid fragments of 2-12 disaccharide length, i.e., about 2000-5000 Da size, to activate dendritic cells.

11. Although the publication of Brand et al is entitled "effects of extracellular matrix on dendritic cells" these authors do not discuss the effects of hyaluronic acid fragments, but rather discuss the role of completely different ECM components, namely, fibronectin and type I and IV collagen, which are proteins and not oligosaccharides.

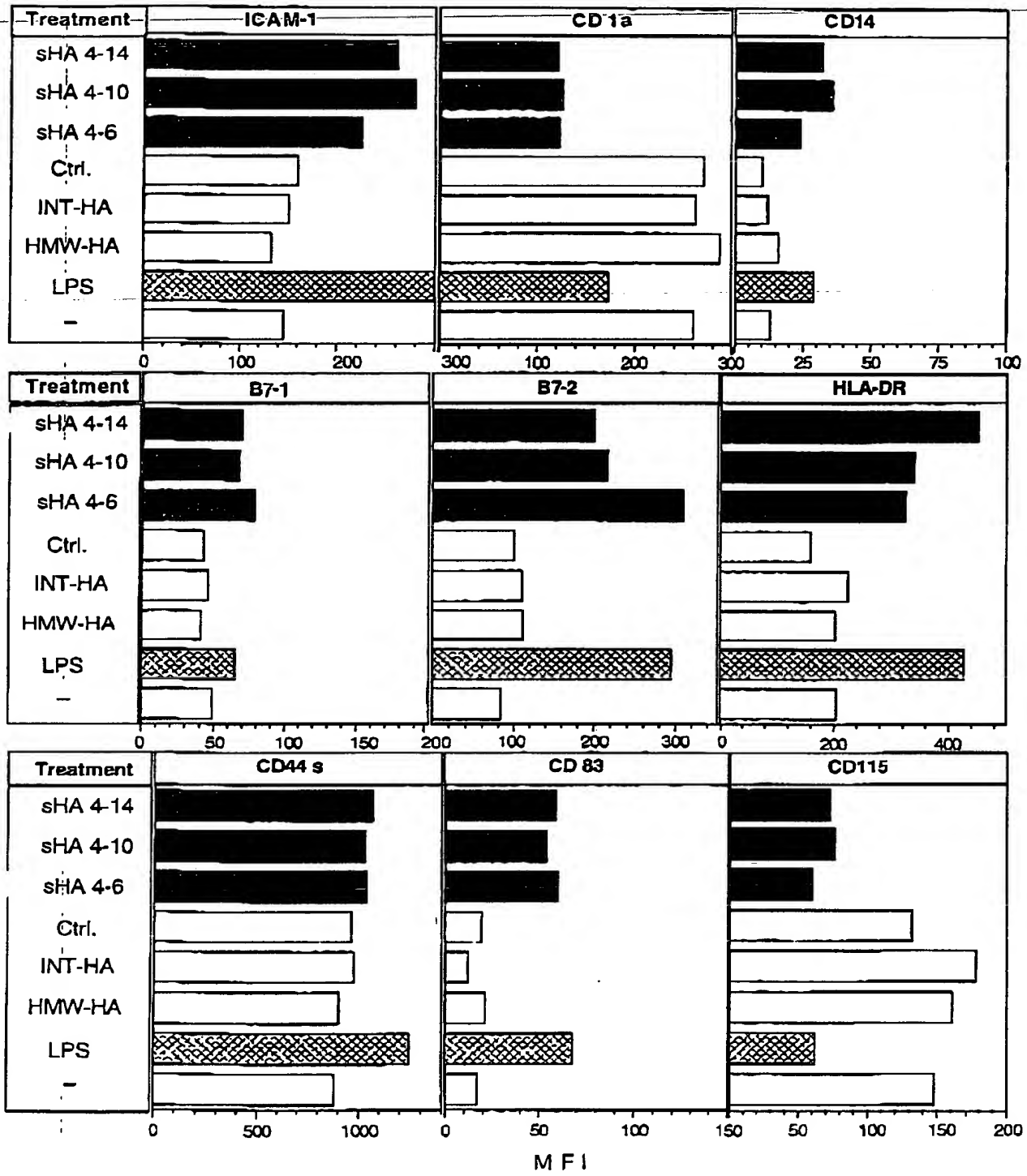
12. Because the extracellular matrix contains a multitude (>500) of components, I strongly disagree that based on the publication of Brand et al., a person of ordinary skill would have been able to use low-molecular weight hyaluronic acid fragments of 2-12 disaccharide length to activate dendritic cells.

13. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above identified application or any patent issued thereon.

Date: May 29 2003

Prof. Dr. Simon

Figure


Treatment
ICAM-1
CD1a
CD14
sHA 4-14
sHA 4-10
sHA 4-6
Ctrl.
INT-HA
HMW-HA
LPS
–
0 100 200
300 100 200
300 25 50 75 100
Treatment
B7-1
B7-2
HLA-DR
sHA 4-14
sHA 4-10
sHA 4-6
Ctrl.
INT-HA
HMW-HA
LPS
–
0 50 100 150 200
100 200 300
0 200 400
Treatment
CD44 s
CD 83
CD115
sHA 4-14
sHA 4-10
sHA 4-6
Ctrl.
INT-HA
HMW-HA
LPS
–
0 500 1000
0 50 100 150
50 100 150 200
M F I